REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Obstetrical and neonatal outcomes from the BEST Trial: single embryo transfer with aneuploidy screening improves outcomes after in vitro fertilization without compromising delivery rates

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OBJECTIVE: We sought to determine whether performing elective single embryo transfer (eSET) after trophectoderm biopsy and rapid aneuploidy screening results in improved obstetrical and neonatal outcomes compared with transferring 2 untested embryos.

STUDY DESIGN: The Blastocyst Euploid Selective Transfer (BEST) Trial enrolled infertile couples with a female partner up to age 42 years who were undergoing in vitro fertilization. They were randomized to receive transfer of a single euploid embryo (eSET) or to the standard of care with transfer of 2 embryos that were not biopsied for aneuploidy screening (untested 2-embryo transfer). Gestational age at delivery, birthweight, and neonatal intensive care unit (NICU) lengths of stay were compared with Mann-Whitney *U*. The risk of preterm delivery, low birthweight, and NICU admission were compared with χ^2.

RESULTS: Among the 175 randomized patients, the delivery rates were similar (69% after euploid eSET vs 72% after untested 2-embryo transfer; $P = .6$) through the fresh cycle and up to 1 frozen transfer, with a dramatic difference in multiple births (1.6% vs 47%; $P < .0001$). The risk of preterm delivery ($P = .03$), low birthweight ($P = .002$), and NICU admission ($P = .04$) were significantly higher after untested 2-embryo transfer. Babies born after untested 2-embryo transfer spent >5 times as many days in the NICU (479 vs 93 days; $P = .03$).

CONCLUSION: By enhancing embryo selection with a validated method of aneuploidy screening, a single euploid embryo with high reproductive potential can be selected for transfer. Using this approach, eSET can be performed without compromising delivery rates and improving the chance of having a healthy, term singleton delivery after in vitro fertilization.

Key words: aneuploidy screening, in vitro fertilization, preimplantation genetic screening, single embryo transfer

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Although a healthy term delivery is the ideal outcome of an in vitro fertilization (IVF) cycle,[1] nearly half of all US babies born after IVF are multiples.[2] Despite improvements with in vitro culture systems, multiple embryo transfer has remained the standard of care due to the inability to predict the reproductive potential of preimplantation embryos. When selecting embryos by the same criteria, it is mathematically impossible that transferring 1 embryo can result in an equal chance of delivery as transferring 2. However, while multiple embryo transfer improves the chance for a delivery after each IVF cycle, it carries a significant risk of multiple gestation conferring increased maternal and neonatal morbidity. Although many infertile couples initially express a desire for twins, most would prefer elective single embryo transfer (eSET) if their chance for a delivery was not compromised.[3]

To perform eSET without compromising per-transfer delivery rates it will be necessary to enhance the method of embryo selection. Having a normal complement of 46 chromosomes is a necessary, but not sufficient, requirement for an embryo to progress to a healthy newborn. Early attempts using fluorescence in situ hybridization to predict the chromosomal status of cleavage-stage embryos and preferentially transfer those predicted to be euploid were unable to improve delivery rates,[4] likely due to a negative impact of the biopsy[5] at the cleavage stage

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and poor accuracy for that technique in this clinical setting.[6] In recent years, however, use of biopsy at the blastocyst stage and more robust assays such as single nucleotide polymorphism arrays and real-time, quantitative polymerase chain reaction (qPCR) to predict the karyotype of embryos have been developed and have demonstrated high accuracy in preclinical validation,[7,8] high negative predictive value,[9] and the ability to improve delivery rates.[10] The improvement in implantation rates was of sufficient magnitude to demonstrate similar delivery rates after transfer of a single euploid embryo compared to transfer of 2 untested embryos,[11] something that had not previously been demonstrated in a randomized controlled trial (RCT).[12]

Since virtually all deliveries after eSET are singletons, we hypothesized that obstetrical and neonatal outcomes would be improved in the group randomized to euploid eSET compared with those receiving transfer of 2 untested embryos. Given the enhanced embryo selection afforded by combining embryo morphology and ploidy status, this would result in an improved chance for a term, singleton delivery.

Materials and Methods

The Blastocyst Euploid Selective Transfer (BEST) Trial was an institutional review board–approved (www.ClinicalTrials.gov registration NCT01408433), randomized, noninferiority trial comparing single embryo transfer after real-time qPCR-based comprehensive chromosome screening to transfer of 2 untested embryos. Patients with an indication for IVF who were <43 years old, with a body mass index ≤30 kg/m^2, and who had an antiMüllerian hormone level of ≥1.2 ng/mL were eligible to participate and informed consent was obtained. Patients were randomized when at least 2 embryos reached the blastocyst stage of development in a 1:1 allocation to receive either euploid eSET or untested 2-embryo transfer. In the euploid eSET group, embryos were tested with a rapid qPCR method of detecting whole-chromosome aneuploidy using assays on each chromosome and providing a result within 4 hours. Details of the rapid qPCR screening methodology have been previously described.[7]

Patients who had at least 2 expanded blastocysts with a discrete inner cell mass by day 5 were eligible for a fresh embryo transfer in the morning on day 6. Those in the euploid eSET group had their embryos biopsied in the afternoon of day 5 with qPCR analysis run overnight. Patients whose embryos were not blastocysts until day 6 or who had contraindications to a fresh transfer (risk of ovarian hyperstimulation syndrome, thin endometrium, premature progesterone elevation), had all of their embryos cryopreserved on day 6 for a future frozen transfer. Those in the euploid eSET group having a frozen transfer had their embryos biopsied on day 6 prior to cryopreservation.

The primary outcome of the study was the ongoing pregnancy rate to a viable gestation after the first embryo transfer, fresh or frozen. A summary of the results has previously been published[11] and the ongoing pregnancy rate after euploid eSET fell within the predetermined 20% noninferiority margin. Patients who received a fresh embryo transfer but did not deliver were encouraged to have a frozen transfer and remain in the group to which they were initially randomized. The current study is an analysis of the final obstetrical and neonatal delivery outcomes through hospital discharge of patients randomized in the BEST Trial after the initial fresh cycle and up to 1 frozen transfer.

Data collection

In compliance with the Centers for Disease Control and Prevention standard of practice for reporting IVF outcomes, patients were contacted after their expected date of confinement and were asked to provide demographic data such as gestational age at delivery, mode of delivery, birthweight, gender, and pregnancy complications. Institutional review board approval was obtained to perform a survey in which patients were queried in more detail about their deliveries, in particular how many days their newborns spent in the neonatal intensive care unit (NICU). Medical records were obtained and the lengths of stay and delivery outcomes were verified. Deliveries occurred at a variety of different hospital settings, both academic and community based, and comparisons between specific rare neonatal complications were not made.

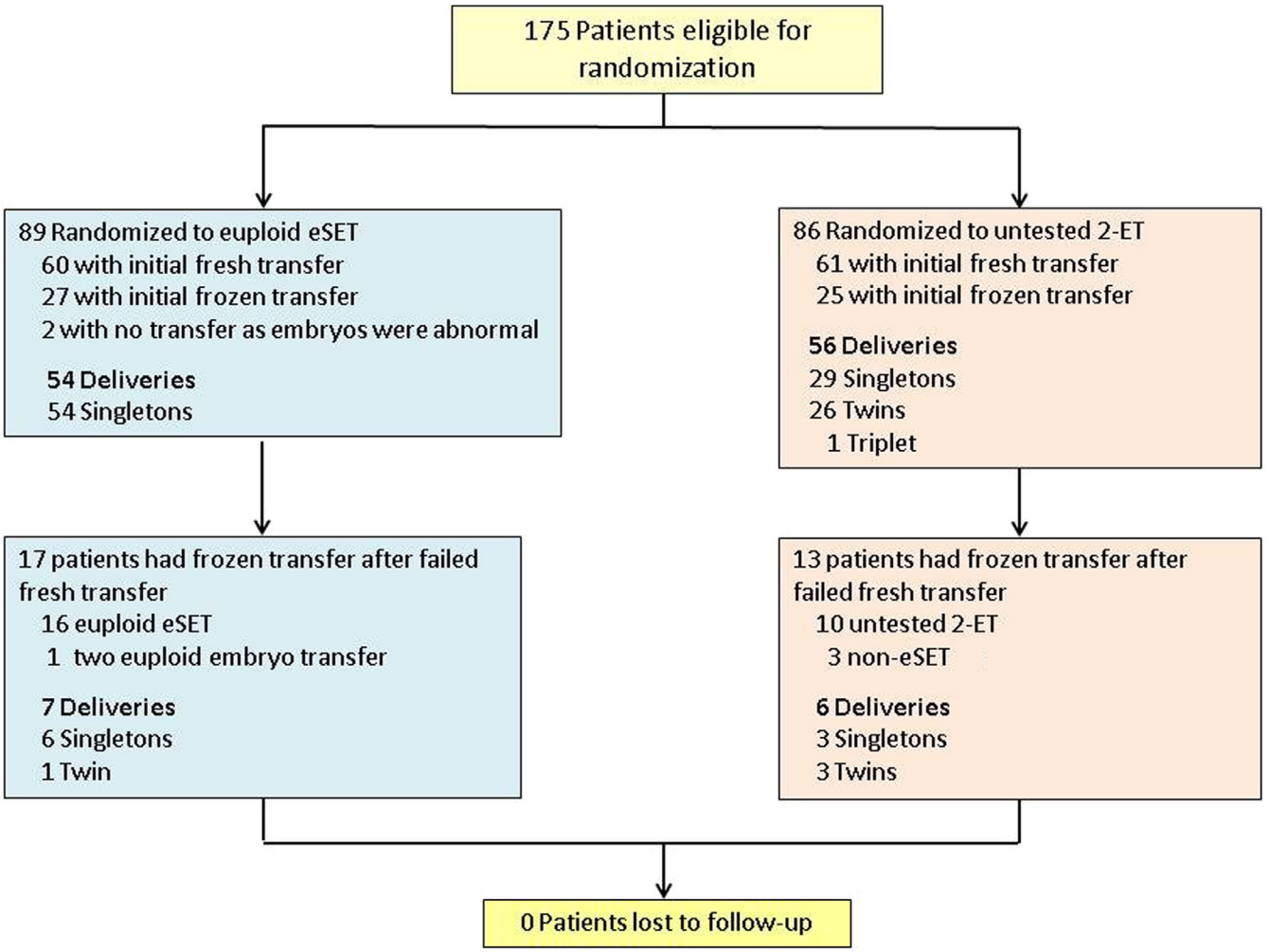
Statistical analysis

Analysis was performed using the intent-to-treat principle such that patients were analyzed based on the group to which they were randomized, regardless of how many embryos were actually transferred. The risk of preterm delivery (<37 weeks), low birthweight (<2500 g), and NICU admission were compared using χ^2. The risk of very low birthweight (<1500 g), a rare outcome, was compared with the Fisher exact test. The birthweight, gestational age at delivery, and length of NICU stay were compared using Mann-Whitney *U*. A *P* value of < .05 was considered statistically significant.

Results

In all, 175 patients were randomized, 89 to the euploid eSET group and 86 to the untested 2-embryo transfer group. The 2 groups were similar in all demographic characteristics with a mean age of 35.1 ± 3.9 and 34.5 ± 4.7 years ($P = .5$), respectively. Patients in each group produced a similar number of blastocysts suitable for transfer with a mean of 5.8 ± 3.6 (range, 2–22) for euploid eSET and 5.3 ± 3.0 (range, 2–18) for untested 2-embryo transfer. In the euploid eSET group 521 blastocysts were biopsied for comprehensive chromosome screening with an aneuploidy rate of 31% (162/521). The proportion of aneuploid embryos increased with increasing age (21% for <35 years old, 34% for 35-37 years old, 56% for 38-40 years old, and 56% for 41-42 years old; $P < .001$). Two patients in the euploid eSET group did not have an embryo transfer as all of their embryos were aneuploid. After the fresh transfer, 34 patients who did not conceive had frozen embryos available and 30 have received subsequent frozen embryo transfers (17 in the euploid eSET group and 13 in the untested 2-embryo transfer group). The cumulative delivery rate after up to 1 frozen transfer was 69% (61/89) after euploid eSET and 72% (62/86) after untested 2-embryo transfer ($P = .6$) (Figure 1). In the

FIGURE 1
Study flow of participants in BEST Trial through up to 1 frozen ET



Patients were randomized when they produced at least 2 blastocysts into 2 groups, comprehensive chromosome screening with euploid elective single ET (eSET) or untested 2-ET. Randomization was stratified for patients who were having fresh or frozen transfer. Patients who did not deliver after their fresh transfer were given opportunity to have additional frozen ET performed.

BEST, Blastocyst Euploid Selective Transfer; *ET*, embryo transfer.

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euploid eSET group, a total of 105 embryos were transferred in 104 transfers compared with 195 embryos in 99 transfers to the untested 2-embryo transfer group. The proportion of transferred embryos that resulted in a live birth was higher after euploid eSET (58% vs 46%; $P = .048$), suggesting an improvement in embryo selection.

The proportion of deliveries that were multiples was dramatically different with 47% (29/62) in the 2-embryo transfer group, including 1 triplet delivery, and 1.6% (1/61) in the euploid eSET group, with 1 dizygotic twin delivery after the patient elected to transfer 2 euploid embryos outside of the study protocol (relative risk, 28.5; 95% confidence interval [CI], 4.01−202.9; $P < .0001$). Per patient randomized, the chance of having a term singleton delivery, the ideal outcome of an IVF cycle, was 60% (53/89) after euploid eSET, which was nearly twice as high as after untested 2-embryo transfer, 31% (27/86) ($P < .001$).

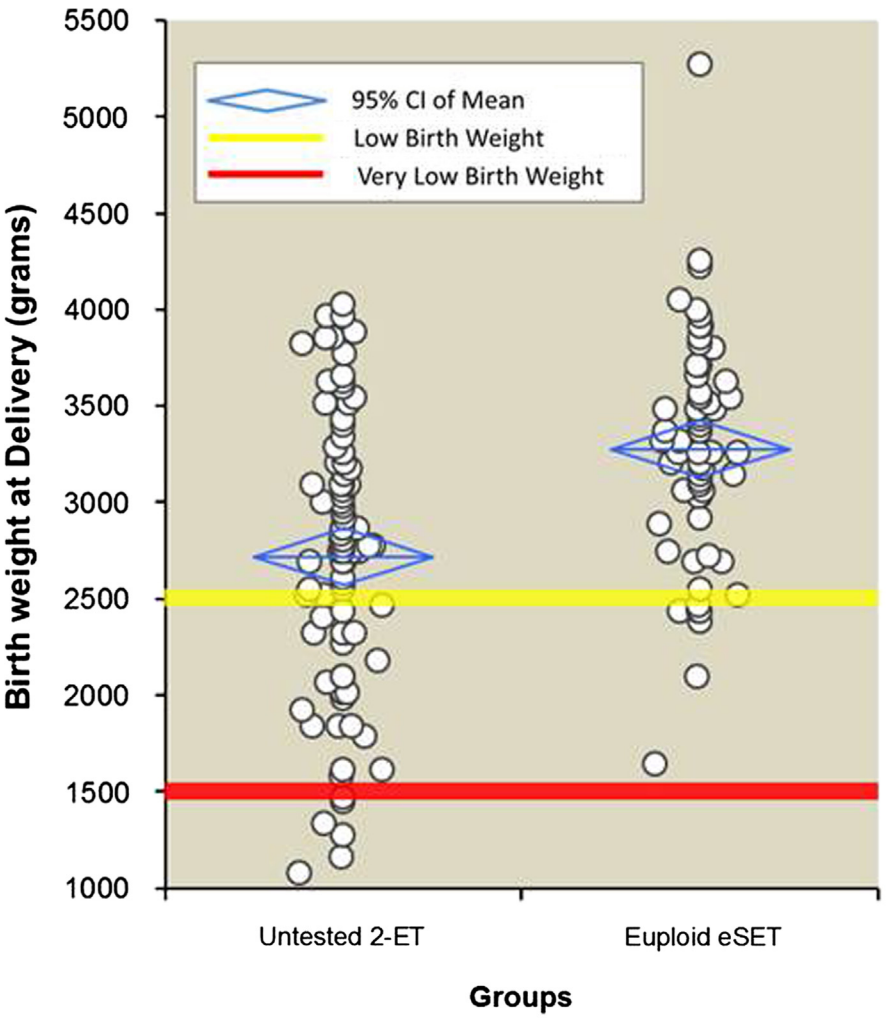
Owing to the difference in multiple gestation, the risk of preterm delivery was higher after untested 2-embryo transfer compared with euploid eSET (29% vs 13%; relative risk, 2.21; 95% CI, 1.04−4.70; $P = .03$). Overall, the untested 2-embryo transfer group delivered at an earlier gestational age (median 38.3 weeks; 95% CI, 37.3−38.6 weeks) than the euploid eSET group (median, 39.0 weeks; 95% CI, 38.9−39.3 weeks) ($P < .001$). The birthweights were lower after untested 2-embryo transfer (median, 2778 vs 3317 g; $P < .0001$). The risk of a newborn being low birthweight (<2500 g) was reduced in the euploid eSET group compared to the untested 2-embryo

transfer group (11% [7/62] vs 33% [30/92]; $P = .002$). Newborns at very low birthweight (<1500 g) are at the most significant risk of long-term morbidity. While this is a rare outcome, there was a trend toward a reduced risk as none of the newborns after euploid eSET were very low birthweight (0% [0/62] vs 7% [6/92]; $P = .08$) (Figure 2).

The risk of having a delivery with a newborn admitted to the NICU was higher in the untested 2-embryo transfer group (26% [16/62] vs 11% [7/61]; $P = .04$). Furthermore, due to more prolonged NICU stays among preterm twins, the babies born after untested 2-embryo transfer spent 5 times as much total time in the NICU compared to those after euploid eSET (479 vs 93 days; $P = .03$) (Figure 3). There were no significant differences in outcomes when analysis was restricted to the singleton deliveries from each group.

Interestingly, the risk of cesarean section was high and not different between the 2 groups (56% after euploid eSET and 61% after 2-embryo transfer; $P = .6$). There was no difference in the rate of birth defects; however, 1 newborn in the euploid eSET group was diagnosed with cri du chat (partial 5p deletion) syndrome after delivery.

FIGURE 2
Birthweights of newborns in each group



Each *circle* represents birthweight in grams of each newborn in untested 2-embryo transfer (ET) and euploid elective single ET (eSET) groups. Risk of low birthweight (<2500 g) was significantly higher after untested 2-ET ($P = .002$) with trend toward higher risk of very low birthweight (<1500 g) ($P = .08$).

CI, confidence interval.

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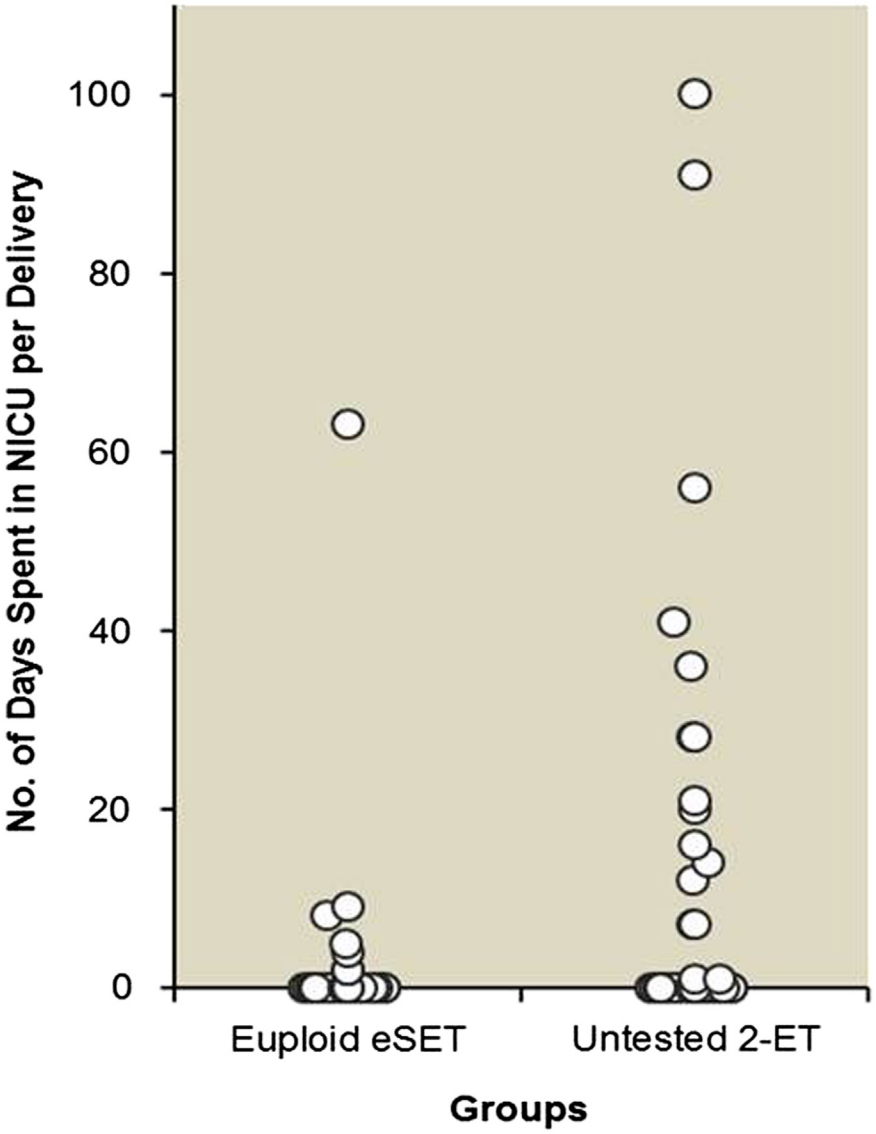
COMMENT

The initial ongoing pregnancy rates from the BEST Trial were previously published and demonstrated that by improving embryo selection using a validated method of aneuploidy screening, such as rapid qPCR, eSET can be performed effectively and maintain excellent delivery rates.[11] The data presented in this study represent the ultimate delivery outcomes after up to 1 frozen transfer for those who did not deliver after the fresh cycle. These obstetrical and neonatal outcomes, not previously reported, demonstrate significant improvements in multiple obstetric and neonatal metrics. The fact that singleton IVF pregnancies are safer than twin pregnancies is well established[13]; the novel aspect of this study is that infertile couples did not compromise their chance for a successful cycle to achieve better obstetrical outcomes. Furthermore, the trial included patients up to age 42 years.

In contrast, most other RCTs of eSET vs 2-embryo transfer were limited to young patients and, even in that good-prognosis population, eSET resulted in inferior delivery rates per transfer.[12,14] Although similar delivery rates have been shown when 1 fresh and 1 frozen single embryo transfer are compared to 1 fresh double-embryo transfer,[15] outside of the RCT setting many patients are likely to elect for a frozen double-embryo transfer after a failed fresh eSET. The ability to perform extended culture and select a single blastocyst, rather than a cleavage-stage embryo, can improve selection for eSET,[16] but cannot result in an equivalent delivery rate as transferring 2 blastocysts. One small RCT comparing the transfer of a single blastocyst to 2 blastocysts failed to show a significantly inferior outcome after eSET[17]; however, that trial only included 48 patients and the 95% CI of the difference included the possibility that eSET was as much as 40% inferior to

FIGURE 3
Length of neonatal intensive care unit stay in each group



Each *circle* in figure represents total number of days spent in neonatal intensive care unit (NICU) for each delivery from untested 2-embryo transfer (ET) and euploid elective single ET (eSET) groups. Risk of NICU admission was higher after untested 2-ET ($P = .04$), and newborns in that group spent more total time in NICU (479 vs 93 days; $P = .03$).

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2-blastocyst transfer. To make eSET more widely practiced in the United States, there is a need to demonstrate improved selection to the point that eSET can rival the excellent outcomes achieved when transferring 2 high-quality blastocysts.

One limitation of the BEST Trial was that it included only patients with a normal ovarian reserve, a normal BMI, and normal endometrial cavities. However, many infertility patients would meet these inclusion criteria and this embryo selection strategy could be applied to many patients undergoing IVF. Additionally, patients meeting these ovarian reserve criteria are the most likely to have at least 2 high-quality embryos to select from and be candidates for eSET. The strategy of extended culture with embryo biopsy and qPCR-based genetic analysis does require additional laboratory procedures with added cost to the IVF cycle. The lack of insurance coverage for the embryo biopsy and genetic testing has been a factor limiting broader acceptance of this screening approach. Although embryo biopsy is an invasive procedure, when performed on trophectoderm at the blastocyst stage, this procedure does not seem to diminish an embryo's chance of implanting and progressing to delivery.[5] Thus, even patients with a favorable prognosis stand to improve their chance of delivering after eSET when the risk of aneuploidy has been dramatically reduced. While the high twin rate suggests that many patients would have delivered if eSET were performed without comprehensive chromosome screening, some patients would have had an aneuploid embryo transferred and thus had a failed transfer.

Furthermore, by empowering the ability to perform eSET even up to age 42 years, trophectoderm biopsy with comprehensive chromosome screening results in improved obstetrical outcomes as evidenced by higher birthweights, lower rates of preterm delivery, lower rates of NICU admission, and shorter NICU stays if admission is required. These differences can likely be attributed to the difference in risk of twins, as the obstetrical and neonatal outcomes between singleton deliveries in both groups were similar. In general twin pregnancies have a higher risk of cesarean section than singletons, but in the current trial there was no difference in the risk of operative delivery between the groups. IVF singleton pregnancies may be at an increased risk of cesarean section, perhaps due to different obstetrical risks in this population.[18] Further studies evaluating long-term pediatric outcomes and the overall cost efficacy of this approach are underway.

The aneuploidy screening that was used in this trial was developed to eliminate the risk of whole-chromosome aneuploidy, the leading cause of failed IVF cycles and clinical miscarriages.[19,20] Using this approach to improve embryo

selection, eSET can be performed without compromising delivery rates. A recent study showed that fetuses with normal karyotypes have up to a 1.7% risk of possessing clinically relevant deletions or duplication.[21] No preimplantation assays have been validated to detect subchromosomal insertions or deletions and do not screen for cri du chat (partial 5p deletion), which 1 newborn was diagnosed with postnatally. Future research using higher resolution arrays or next-generation sequencing may be able to incorporate preimplantation screening for clinically relevant insertions and deletions.

In summary, by culturing embryos to the blastocyst stage, performing a trophectoderm biopsy, and amplifying DNA with qPCR assays on each chromosome, a single euploid blastocyst with high reproductive potential can be selected for transfer. This paradigm eliminates the risk of multizygotic multiple gestation and increases the chance for a healthy, term singleton delivery without requiring patients to undergo an increased number of failed cycles. The improved obstetrical and neonatal outcomes suggest this approach may become the standard of care for infertile couples requiring IVF. ■

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